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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Application of

Tomoyasu Ishikawa et al.

Serial No. : 09/555,949 Group Art Unit : Art Unit 1624

Filed : June 6, 2000 Examiner : Mark L. Berch

For : PHOSPHONOCEPHEM DERIVATIVES, THEIR PRODUCTION AND USE

DECLARATION UNDER 37 CFR § 1.132

I, HIDETOSHI HORIBE, a citizen of Japan, residing at 1-3-7-507,Nishi-Izumigaoka, Toyonaka City, Osaka, Japan, declare:

That I was born on October 3, 1953 in Nagano, Japan and graduated from Doctoral Course, Faculty of Pharmacy, The Chiba University in Chiba, 1983 and was awarded Ph.D. degree

That I have been employed by Takeda Chemical Industries, Ltd., Japan since April, 1983 and have been engaged in research work in the field of pharmaceutical technology;

That I am a Research Manager of Drug Analysis & Pharmacokinetics Research Laboratories, Pharmaceutical Research Division of the said company;

That I am a member of The Pharmaceutical Society of Japan and The Academy of Pharmaceutical Science and Technology, Japan;

That, in order to make clear that the present invention has an unexpected result, the following experiment was conducted by myself or specialized technicians under my direct guidance and supervision.

I hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United Stated Code and that such willful false statements may jeopardise the validity of the application or any patent issued therefrom.

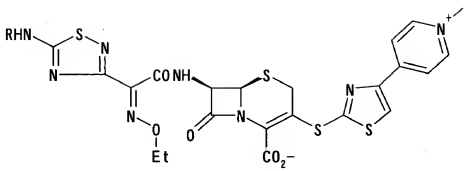
Signed this 3rd day of August, 2001

Hidetoshi Horibe

Hidetoshi Horibe

Experiment

1. Test compound



Compound A : R = $PO(OH)_2$

7β-[2(Z)-ethoxyimino-2-(5-phosphonoamino-1,2,4-thiadiazol-3-yl)acetamido]-3-[4-(1-methyl-4-pyridinio)-2-thiazolylthio]-3-cephem-4-carboxylate

Compound B : R = H

7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(Z)-ethoxyiminoacetamido]-3-[4-(1-methyl-4-pyridinio)-2-thiazolylthio]-3-cephem-4-carboxylate

2. Method

Approximately 2mg of Compound A was accurately weighed and dissolved in 20mL of Britton-Robinson's buffer solution (pH 7) to make a sample solution of 100 μg/mL. The sample solution was stored at 25℃ and the remaining Compound A was measured periodically by the following HPLC condition. Equally, a sample solution of Compound B of 100 μg/mL was prepared, stored at 25℃ and the remaining Compound B was measured periodically by the following HPLC condition.

HPLC condition
Detector: UV254nm

Column:	YMC ProC18 150 × 4.6mm
Mobile phase A:	0.05mol/L $AcONH_4$/MeCN (960 : 65)
Mobile phase B:	0.05mol/L $AcONH_4$/MeCN (960 : 4650)
Gradient program:	0→20min B:0→5%, 20→40min B:5→20%, 40→50min B:20→40%
Column temp.:	constant temperature about 25℃
Flow rate:	1mL/min
Injection volume:	10 μ L
Retention time:	Compound A : about 20min, Compound B: about 40min

3. Result

Table I shows the analytical result. Both of Compound A and Compound B were gradually decomposed according to pseudo-first order kinetics at pH 7, at 25℃. The rate constants (k) and the half-life ($t_{0.5}$) of Compound A and Compound B were calculated as shown in Table I.

Compound A was more stable in the solution at pH 7 than Compound B.

Table I Stability of Compound A and Compound B in Aqueous Solutions at pH 7 at 25℃

Time	% of Initial	
(hours)	Compound A	Compound B
0	100	100
4	99.2	97.9
8	98.3	96.6
12	96.9	94.7
16	94.7	93.4
20	93.8	92.3
24	93.7	90.7
48	88.7	84.2
k_{obs} X 10^3 (h^{-1})	2.6	3.5
$t_{0.5}$ (h)	266	196